

RESEARCH ARTICLES

Thiopurine Methyltransferase in Thai Population

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Abstract

The cytosolic enzyme, thiopurine methyltransferase (TPMT) catalyzes the S-methylation of aromatic and heterocyclic sulfhydryl compounds such as 6-mercaptopurine. Genetic regulation of TPMT activity is associated with large interindividual variations in thiopurine toxicity and efficacy. Assessment of TPMP genotype/phenotype will increase rational outcome of individual drug therapy. In the present investigation, TPMT activity was measured by the radiochemical assay from blood samples of 539 randomly selected Thai subjects. The genetic polymorphism of TPMT was trimodal distribution, TPMT activity ranged from 0 - 23.5 Units/ml RBC with the average of 6.91 ± 3.68 Units/ml RBC. Most of the Thai subjects were intermediate metabolizers (95.35 %), followed by high (3.75 %) and low (0.93%) metabolizers. Gender, blood groups and smoking had no effect on TPMT activity.

Key words : thiopurine methyltransferase, genetic polymorphism, 6-mercaptopurine.

สมรรถนะของเอนไซม์ไธโอพิวรีนเมทิลทรานสเฟอเรสในคนไทย

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บทคัดย่อ

เอนไซม์ไธโอพิวรีนเมทิลทรานสเฟอเรส (TPMT) กระตุ้นปฏิกิริยา S-methylation ของสารประกอบ aromatic และ heterocyclic sulfhydryl เช่น 6-mercaptopurine การควบคุมทางพันธุกรรมของเอนไซม์ TPMT เกี่ยวข้องกับความแตกต่างอย่างมากในแต่ละบุคคล ที่มีผลต่อการเกิดพิษและการรักษาของยา thiopurines การประเมินหา genotype/phenotype ของ TPMT จะช่วยเพิ่มประสิทธิภาพการรักษาด้วยยาเฉพาะบุคคล การวิจัยครั้งนี้ได้ทำการวัดสมรรถนะของเอนไซม์ TPMT โดยการใช้ radiochemical assay ในเม็ดเลือดแดงของคนไทยจำนวน 539 ตัวอย่างจากการคัดเลือกแบบสุ่ม พบว่า genetic polymorphism ของ TPMT มีการกระจายของประชากรเป็น 3 กลุ่ม สมรรถนะของเอนไซม์อยู่ระหว่าง 0 - 23.5 Units/ml RBC ค่าเฉลี่ย 6.91 ± 3.68 Units/ml RBC คนไทยส่วนใหญ่มีสมรรถนะของเอนไซม์ปานกลาง (95.35%) ตามด้วยสมรรถนะของเอนไซม์สูง (3.75%) และสมรรถนะของเอนไซม์ต่ำ (0.93%) เพศ หมู่เลือด และการสูบบุหรี่ ไม่มีผลต่อสมรรถนะของเอนไซม์ TPMT

คำสำคัญ : thiopurine methyltransferase, genetic polymorphism, 6-mercaptopurine.

Introduction

The cytosolic enzyme, thiopurine methyltransferase (TPMT, EC 2.1.1.67) catalyzes S-methylation of aromatic and heterocyclic sulfhydryl compounds such as anticancer drugs 6-mercaptopurine, 6-thioguanine and immunosuppressant azathioprine^{1,2,3}. TPMT activity exhibits genetic polymorphism, in white subject approximately 90 % are homozygous with high activity, 11 % are heterozygous with intermediate activity and 1 in 300 individuals inherit TPMT deficiency as an autosomal recessive trait^{4,5}. This common genetic polymorphism is responsible for interindividual variations in thiopurine toxicity and therapeutic efficacy⁶. Patients with low TPMT activity develop severe myelosuppression with standard doses of these drugs while patients with high TPMT activity are undertreated⁷.

The active gene of TPMT is located on chromosome 6 with approximately 34 kb in length and consisted of ten exons and nine introns. The wild type allele is TPMT*1. At least 8 mutant alleles have been identified for low enzyme activity. These mutant alleles have interethnic variability with different frequency and pattern. In Chinese, West African, Kenyan and Japanese the mutant allele is TPMT*3C. TPMT*3A is found in Caucasian and South-West Asian and TPMT*6 in Korean^{8,9,10}. All mutant alleles in South East Asian are now identified as TPMT*3C¹¹.

TPMT activity is normally detected in red blood cells, showing high correlation with relative TPMT levels in other tissues or cells including kidney, liver, lymphocytes and leukemic blast cells^{2,4,12,13}. The radiochemical assay by Weinshilboum et al⁴ is a well known method in determining TPMT activity. The product of S-methylation, 6-methylmercaptopurine is measured using 6-mercaptopurine as substrate and S-adenosylmethionine (SAM) as the methyl donor. One unit of enzyme activity represents the formation of 6-methylmercaptopurine in nanomoles per one milliliter of red blood cells^{2,4}.

There is very little information about TPMT activity in Thai population. Therefore the frequency of TPMT phenotype was studied in the present investigation.

Material and method

Subjects

A total of 539 blood samples from healthy blood donors at National Blood Center, Thai Red Cross Society were used for TPMT activity study. The exclusion criteria were hepatic, renal and other chronic diseases. The demographic data was collected from each blood donor including sex, age, blood group, smoker/non-smoker, and current medical problems.

Blood samples: preparation of lysates

Samples were prepared as previously described by Weinshilboum et al⁴. Aliquot of 3 ml whole blood collected in heparinized tube was centrifuged at 800g for 10 min at 4°C to isolate red cells. Plasma, leukocytes and the upper layer of erythrocytes were removed. After washing the pellet twice with 1.8 ml 0.9% normal saline and centrifugation for 10 min at 800g, supernatant was discarded, 4.8 ml iced cold water was added to the pellet to lyse the red cells. Erythrocyte lysate was centrifuged at 13,000g for 10 min at 4°C. The supernatant was kept at -80°C until analysis.

TPMT assay

Potassium phosphate buffer, 25 µl (150 mM, pH 7.5) and either 5 µl of 6-mercaptopurine (18 mg/ml in DMSO) or 5 µl of DMSO were added to 100 µl aliquot of erythrocyte lysate. The reaction was initiated by adding 25 µl of a freshly prepared cocktail containing ¹⁴C-methionine 12.5 x 10⁻⁶ M, non-reactive S-adenosyl-L-methionine HCl 12.5 x 10⁻⁶ M, dithiotreitol 10⁻³ M and allopurinol 5 x 10⁻⁵ M. The reaction mixture was incubated and gently shaken for 1 h at 37°C in a shaker water bath. To terminate the

reaction, 0.5 ml boric acid buffer, pH 10 and 5 ml of 20% isoamyl alcohol in toluene were added. After vigorous agitation for 10 seconds, the mixture was centrifuged at 700g for 10 min at 4°C. A 3.5 ml aliquot of the supernatant (organic phase) was added to the scintillation counting vial, to which containing 1 ml of absolute ethanol and 10 ml of toluene fluor. Radioactivity in the scintillation vials were counted in the scintillation counter for 10 min. The TPMT activity was expressed as nanomoles of 6-methylmercaptapurine formed per hour per ml of packed red cells (hematocrit) or Units/ml RBC.

Statistical analysis

Data was presented as mean \pm SD. Probit analysis was used for frequency distribution. Data comparison was tested by one way ANOVA using Duncan's New Multiple Range test.

Result

The correlation between experiment was carried out using same reference

enzyme together with other test samples. The average TPMT activity between experiment of the reference enzyme was 6.54 ± 0.24 Units/ml RBC with C.V. of 3.7%.

TPMT activity was studied from 539 blood samples of healthy 438 men and 101 women with age range 17-64 years (average 35.5 years). Enzyme activity varied from 0 to 23.5 Units/ml RBC. The mean value was 6.91 ± 3.68 Units/ml RBC. There were no differences of TPMT activity in different gender, blood groups or smoker/non-smoker (Table 1).

The distribution of TPMT activity was trimodal (Fig. 1). With probit analysis (Fig. 2) the high enzyme activity were 16.7–23.5 Units/ml RBC, accounted for 3.72% of population studied, while 95.35% carried intermediate enzyme activity ranging from 1.3 to 16.6 Units/ml RBC and 0.93 % with low enzyme activity of 0-1.2 Units/ml RBC.

Table 1 TPMT activity from 539 healthy blood donors with age range 17-64 years (average 35.5 years)

Demographic data	Numbers	TPMT activity*(Units/ml RBC)		
		Average	Maximum	Minimum
Sex: Men	438	6.93 ± 3.55	23.53	0.00
Women	101	6.91 ± 4.11	22.30	0.15
Blood groups: A	88	6.80 ± 3.76	19.78	0.00
B	177	6.73 ± 3.59	22.32	0.14
O	257	7.10 ± 3.78	23.53	0.12
AB	17	6.24 ± 2.66	13.23	1.00
Smoking: Smoker	42	6.67 ± 4.21	22.27	0.13
Non-smoker	497	6.93 ± 3.62	22.53	0.00
Total subjects	539	6.91 ± 3.68	23.53	0.00

* Mean \pm SD

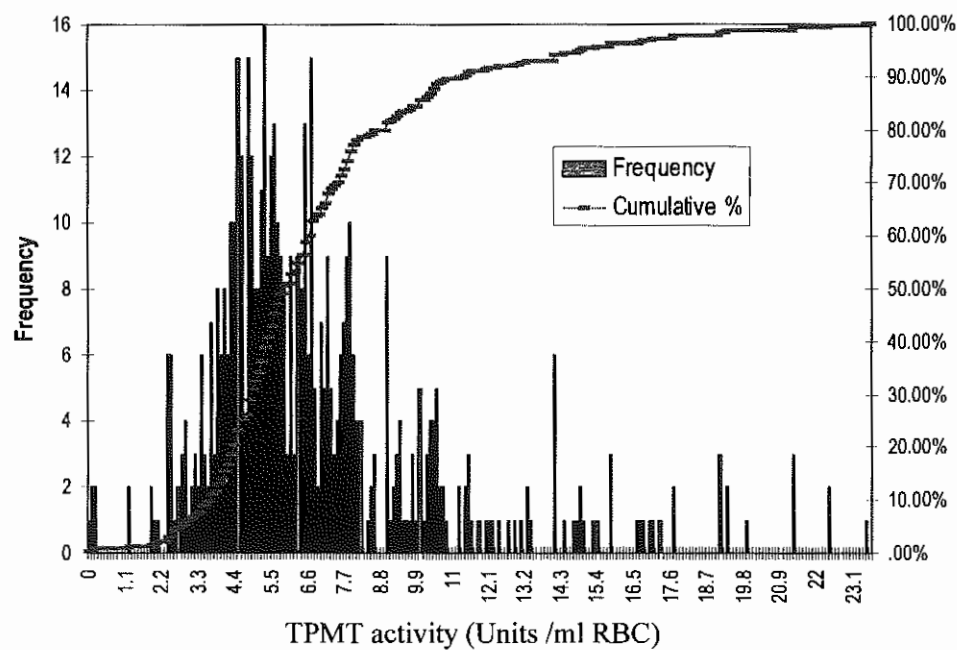


Figure 1 The relationship between frequency and TPMT activity in Thai population

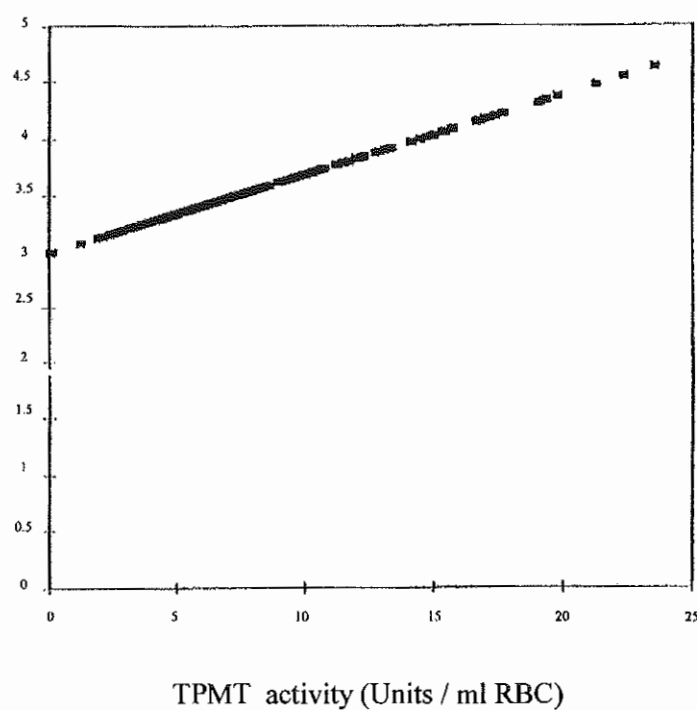


Figure 2 Probit graph of the relationship between frequency and TPMT activity in Thai population..

Discussion and conclusion

From present investigation, most of Thai carried intermediate TPMT activity which means that Thai may be prone to thiopurine toxicity, especially children treating with 6-mercaptopurine in acute lymphoblastic leukemia. Determination of TPMT genotype/phenotype before antileukemic therapy may be practical and may have clinical relevance.

Frequency of TPMT genotype in Thai children with acute leukemia (75 patients)¹⁴ and 200 healthy Northeastern Thai subjects¹⁵ reported to be the same as Caucasian population. The variant allele was TPMT*3C.

Therefore the correlation between genotype and phenotype assessment in Thai population should be performed to verify the use of either TPMT genotype or phenotype in minimizing toxicity and maximizing efficacy of thiopurine drug therapy.

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Reference

1. Remy CN. Metabolism of thiopyrimidine and thiopurine : S-methylation with S-adenosyl methionine transmethylease and catabolism in mammalian tissue. *J Biol Chem* 1963; 238: 1078-1084.
2. Woodson LC and Weinshilboum RM. Human kidney thiopurine methyltransferase: purification and biochemical properties. *Biochem Pharmacol* 1983; 32: 819-826.
3. Ames MM, Selassie CP and Dulczynska M. Prognosis significance of hepatotoxicity during maintenance chemotherapy for childhood acute lymphoblastic leukemia. *Br J Cancer* 1990; 61: 767-772.
4. Weinshilboum RM and Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Human Genetic* 1980; 32: 652-662.
5. Woodson LC, Dunnette JH and Weinshilboum RM. Pharmacogenetics of human thiopurine methyltransferase: kidney-erythrocyte correlation and immunotitration studies. *J Pharmacol Exp Ther* 1982; 222: 174-181.
6. Aarbakke J, Janka-Schaub G and Elion GB. Thiopurine biology and pharmacology. *TiPs* 1997; 18: 3-7.
7. Yan L, Zhang S, Eiff B et al. Thiopurine methyltransferase polymorphic tandem repeat: genotype-phenotype correlation analysis. *Clin Pharmacol Ther* 2000; 68: 210-219.
8. Loennechen T, Yates CR and Fessing NY et al. Isolation of a human thiopurine S-methyltransferase (TPMT) complementary DNA with a single nucleotide transition A719G (TPMT*3C) and its association with less of TPMT protein and catalytic activity in humans. *Clin Pharmacol Ther* 1998; 64: 46-51.
9. Ando M, Ando Y, Hasegawa Y et al. Genetic polymorphisms of thiopurine S-methyltransferase and 6-mercaptopurine toxicity in Japanese children with acute lymphoblastic leukemia. *Pharmacogenetics* 2001; 11: 269-273.
10. Kham SKY, Tan PL, Tay AHN et al. Thiopurine methyltransferase polymorphisms in a multiracial Asian population and children with acute lymphoblastic leukemia. *J Pediatric Hematology/Oncology* 2002; 24: 353-359.
11. McLeod HL. The thiopurine methyltransferase gene locus-implications for clinical pharmacogenomics. *Pharmacogenomics* 2002; 3: 89-98.
12. Szumlanski CL, Honchel R and Scott MC et al. Human liver thiopurine methyltransferase pharmacogenetics: biochemical properties, liver-erythrocyte correlation and presence of isozymes. *Pharmacogenetics* 1992; 2: 148-159.
13. Otterness D, Szumlanski C, Lennard L et al. Human thiopurine methyltransferase pharmacogenetics: gene sequence polymorphisms. *Clin Pharmacol Ther* 1997; 62: 60-73.
14. Hongeng S, Sasanakul W, Chuansumrit A et al. Frequency of thiopurine S-methyltransferase genetic variation in Thai children with acute leukemia. *Med Pediatric Oncology* 2000; 35: 410-414.
15. Srimartpirom S, Tassaneeyakul W, Kukongwiriyan V et al. Genetic polymorphism of thiopurine S-methyltransferase in a Northeastern Thai population. *Thai J pharmacol* 2002; 24 suppl 1: S135.